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Training, Muscle Fatigue, and Stress Fractures

Annual Report

Clinton T. Rubin, Ph.D.

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Abstract

The sharp rise in physical demands initiated during basic training all too frequently will precipitate stress fractures in the lower appendicular skeleton of new recruits. During peacetime, stress fractures are by far the most common physical injury in the military population, and are responsible for the military's greatest drain of both lost recruit time and medical expense. The objective of this three year research program is to study the etiology of the stress fracture lesion, and isolate any aspects of a physical regime which may exacerbate this condition. To perform this mission, we are pursuing two parallel experimental protocols: a) the effect of muscle fatigue on bone strain distributic and, b) the effect of repetitive cyclic loading on bone remodeling. The summation of these two protocols has already provided unique insight towards the effect of new strain regimens on skeletal remodeling, and almost certainly will isolate and identify specific activities which may accelerate the pathogenesis of this condition.

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Summary

The progress through the second year of this project includes: The continued development of a unique protocol in eight thoroughbred horses which will quantify the effect of fatigue and uncoordinated muscle activity on the distribution, magnitude and rate of change of strains developed *in vivo* within the third metacarpal (cannon bone). It is this bone in the horse that suffers most often from the stress fracture pathology, creating a huge medical and financial burden on the racing industry, and similar to the consequences the military endures. The methodology developed for this protocol includes bone-bonded rosette strain gauges, liquid metal strain gauges attached to tendon, surface and muscle embedded electrodes, heart and ventilation frequencies, blood lactate production, maximum oxygen consumption, and carbon dioxide production. We are also developing the means to make these recordings in the field via transmitter, to measure the physical stresses engendered during more "natural" conditions, and thus exacerbate a less controlled environment.

We are nearing completion of a rigorous analysis of the strain measured from the cannon bone during extreme activities. This analysis includes not only longitudinal strain distribution, but accounts for shear, bending, torsion and strain energy density. As a result of this extensive engineering analysis of the cannon bone, we have been able to identify a correlation between the quadrant of the cortex that is most susceptible to the lesion (Antero-lateral), and the strain-energy density of the bone. Surprisingly, the lesion occurs where the strain energy

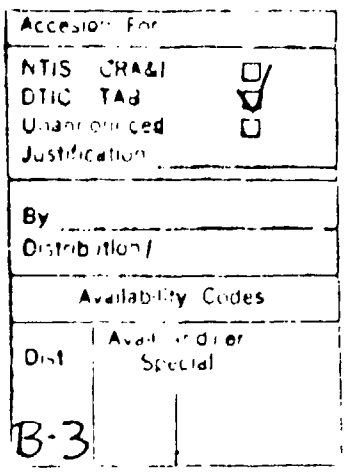
density is the smallest.

In addition, the protocol using the in vivo ulna preparation has progressed, to determine the remodeling response to repetitive cyclic loading. The analysis of the resultant pathology is currently being developed using Computer Aided Tomography (CAT), Electron Microscopy (EM), Finite Element Modeling (FEM), Cellular Kinetic Stereology (CKS) and standard histologic techniques. We are developing assays for ash and collagen content, as well as identifying "activated" cell surfaces using radioimmunochemistry and histochemistry. These techniques are currently being applied to not only the mature male turkey ulna model, but to better understand the influence of physical parameters on the immature skeleton, we are instigating a series of experiments whereby cyclic loads will be applied to the growing turkey. This should magnify any distinctions between the stress fracture etiology between the adult and non-adult skeleton (i.e., is the growing skeleton more susceptible to the lesion?). We are also currently looking into the role of frequency (vibration, skeletal resonance) on the control of remodeling and the acceleration of damage.

In support of our findings in the equine model for stress fractures, the lesion which appears on the mature turkey when following high cyclic loading is located at the neutral axis of normalized stress - where the longitudinal strains are smallest -rather than on the cortices where the strain is greatest.

Foreword

In conducting research using animals, the investigators adhered to the "Guide for the Care and use of Laboratory Animals", prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985). Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.



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Introduction

Stress fractures are the leading cause of time lost in basic training since, for adequate healing, they require at least 6-8 weeks of rest without weight bearing. Attempts to reduce the incidence of this debilitating injury have met with varying degrees of success. This is largely due to our limited understanding of the mechanical factors (stress, strain, strain rate, strain distribution, number of strain repetitions, etc.) which are actually responsible for causing the structural damage in the bone tissue. The objective of the series of experiments reported here is to determine to what extent repetitive cyclic loading applied at levels which would be engendered during basic training may be 'perceived' by the bone as a stimulus to internal bone remodeling, the precursor to the stress fracture lesion.

The conventional view of stress fractures is that increased functional activity engenders extracellular microdamage within strategic areas of the cortex. The damage would accumulate until the structural integrity of the bone is compromised. This scenario is not unlike the bending of a paperclip until sufficient 'microdamage' builds up and the wire breaks, hence the stress fracture pseudonym: "Fatigue fracture". In the skeleton, this sequence could be aggravated by the relatively slow remodeling process, inherent to bone tissue. If bone is incapable of repairing or replacing those areas of the cortex which have been damaged before loading continues, mechanical fatigue and ultimately structural failure is a predominant possibility.

In this experimental protocol, two hypotheses have been proposed:

a) changes in muscle firing patterns (recruitment due to metabolic fatigue) will alter the strain distribution through the bone, and

b) change in strain distribution within the bone will stimulate an adaptive remodeling process.

This report will summarize the experiments lesion continued through the second year of study.

Methodology and Results

a) Locomotion experiments:

The primary objective of this series of experiments is to establish a protocol capable of monitoring changes in muscle recruitment patterns in the forelimb of an exercising horse, and correlate these changes with any simultaneous alterations in the distribution of strain across the third metacarpal (MCIII). The investigators contributing to this protocol include Drs. Rubin, Seeherman, McLeod and Gray. Technical support was provided by Ms. Kinney and Ms. Craig.

Following acclimation training on the treadmill, eight horses were surgically instrumented, under a general halothane anesthesia and under aseptic conditions, in the following manner:

Through a medial and a cranio-lateral incision, three, three element rosette strain gauges were attached to the surface of the medial, cranial, and lateral aspects of the diaphyseal midshaft of the cannon bone (MCIII). This was performed by removal of the periosteum (scraping of one

square centimeter), and degreasing the bone with anhydrous ether. The gauges are attached using 2-isobutyl cyanoacrylate, and strain relief flanges are screwed to the bone, approximately 2 c.m. from the gauge site, to minimize tension on the lead wires. All wounds are sutured closed, and the limb bandaged. The trauma to the animal is minimal, allowing for a full exercise protocol and one day post-op.

i) EMG activity: On the third day post-op, the animal has surface EMG electrodes glued to the skin surface over the extensor radialis and common digital extensor muscle groups. We had originally used imbedded plugs, but the movement inherent in high speed locomotion caused continual breaking of the fine wires which constitute this implant. The development of this work was performed by Dr. Kenneth McLeod, a Research Assistant Professor in our laboratory with a Ph.D. in electrical engineering awarded from M.I.T. Eighty percent of Dr. McLeod's time is oriented towards the Army project.

Surface EMG of the CDE in the trotting at 2.5 m/s on a flat surface was examined before and after a strenuous exercise session (7 minutes at 2.5 m/s at a 10% incline). Spectral analysis of the linear envelope of the rectified EMG demonstrated that spectral densities were uniformly ~60% higher in the fatigued state. Spectral analysis of the raw EMG revealed a doubling in the activity of high conduction velocity motor units and no change in the activity of the lower velocity units. Since the trotting rates are constant, these results suggest either that greater electrical excitation is required to achieve the

same muscle force exerted by the CDE, or that an increase in force generated by the CDE is required to compensate for fatigue induced in other muscle groups. If the latter is true, then fatigue induced loading of the skeleton may in fact generate a different 'new' strain distribution on the bone, which in turn may stimulate active adaptive remodeling within the bone. This condition is considered below, in the strain gauge section.

ii) LMSG activity: we have tried to quantitate the magnitude of force generated within specific muscle groups, and any change in magnitude engendered by fatigue. This objective required the development of a small implantable transducer capable of monitoring some component of muscle activity, other than the more qualitative limitations set by EMG measurement. We pursued this objective through the development of a commercially available liquid metal strain gauge. These gauges are thin silastic tubes, approximately 1.5 mm in diameter and 10 mm in length, which are filled with mercury. Lead wires are attached to the ends of the tube such that changes in resistance across the tube can be monitored as the tube length changes. Small rectangles of PVC (3 x 8 mm) are glued to each end of the gauge to serve as anchoring points to the tendon. Under general anesthesia (as above) and using aseptic technique, one gauge is sutured to the deep digital flexor tendon (DDFT), and one to either the lateral digital extensor tendon (LDET). The gauges are prestreched and attached to the tendon bodies by suturing through holes in the PVC flanges. The two lead wires are led subcutaneously, and passed through the skin proximal to the carpal joint. The wounds are then sutured closed. One day post-operatively, the lead wires

are connected to a wheatstone bride amplifier, and liquid strain gauge outputs recorded while the animal runs on the motorized treadmill.

The data generated by these LMSG gauges has proven limited. To evaluate the actual magnitude of the forces generated by these muscle groups, it is critical to calibrate the gauges in situ (i.e., load control tension tests of the tendons post-mortem, while the originally implanted gauge is still functional). Unfortunately, no gauge has remained functional through the entire experimental period.

Although the LMSG technique was pursued through the second year, no results were considered reliable or repeatable enough to generate confidence in the protocol. While the LMSGs appear to work well in animals of smaller mass (dogs, sheep), the horses' movement is just too violent, creating an inescapable resonance in the gauge. We believe it is this inertial stretching on impact that diminishes the gauges reliability. The further development of this protocol has been temporarily suspended.

iii) Bone strain activity: The bone-bonded strain gauge technique is working well. The cannon bones of eight horses have been instrumented, and data recovered from all animals. In addition, remote recording techniques are being developed to obtain data from the field, rather than being limited to the extreme of controlled conditions, the motorized treadmill. In the eighth animal, we were successful in determining gage locations and geometric properties of the canon bone in vivo using radiographs and computer aided tomography (CAT scanning) respectively. This will allow us, in the future, to use more fit

and faster animals, as the strain gages can now be removed under anesthesia, and the animals returned to pasture or racing.

Three three-element rosette gauges, generating nine channels of strain data from the bone, give direct indications of strain from the bone's surface. The "raw" strain from each gauge is analyzed to give principal strains from the three gauge locations, and therefore distributions of principal strain across the bone can be determined. We are monitoring this distribution such that any changes which may occur with exercise will be observed.

The analysis has been limited to strains along the bone's material coordinate system (longitudinal) and, therefore, may 'overlook' other relevant strain parameters (i.e., shear strain). Dr. Martha Gray, a post-doctoral fellow for 1987 in the laboratory, had begun the development of this analysis to account for these other strain parameters. Unfortunately, Dr. Gray accepted a position in the department of Bioengineering at Harvard Medical School, and has left the lab. Mr. Ramiah Vasu, with a M.S. in medical engineering from the University of Washington is continuing this analysis.

The new analysis has provided a body of unique and very exciting data. Rather than being limited to longitudinal (normal) strain, or the principal strain distribution, we can now calculate the distribution of shear strain, and strain energy density, an index of the greatest "work" done by the bone material. This is an important advancement in our analysis, as it unveils several additional mechanical parameters that previously we had no access to. The analysis provides a detailed mechanical profile of the bone through a cortical cross section,

at any point in the stride and through a series of strides at any given speed. For that specific point in the stride (e.g. 41% into stance phase), a series of twenty strides are interpolated to create a single representative average stride. Principal strains are calculated at each rosette gauge, and a distribution of normal and principal strains across the cortex determined using simple beam theory.

The analysis will now also calculate the shear strains, and their distribution, through the stride. To our knowledge, we are the first lab in the world with the capacity to calculate in vivo shear distribution, (three rosette strain gauges required) and determine the points of highest and lowest shear, and their correlation to peak principal strains. By improving our understanding of the mechanical milieu of the bone, we will establish a better means of identifying its modes of failure during high cyclic activities.

The analysis will also determine the strain energy density, which represents those areas of the cortex which undergo the greatest level of "work". This is important as high levels of shear may correspond to low levels of normal strain, and vice versa. This calculation will identify those areas that see the greatest (and least) strain distortions during activity.

As examples of this calculation, we can show the distribution of normal strain generated during a trot at 6 meters/sec. At the peak strain magnitude of the stance phase, the normal strain distribution shows a strain magnitude of 2019 microstrain on the medial cortex (fig. 1a), with the neutral axis of strain running through the lateral cortex. Given only this plot, we are forced to consider the strains represented by these

normal strains, i.e., strains at the neutral axis are zero. However, this calculation is not sensitive to the shearing (or tearing) deformation. With the new calculation, a shear distribution, with relative direction, can be calculated (fig. 1b). This demonstrates, at each location within the cortex, the levels of shear that the bone is subjected to. In this case, shear strains of 1889 microstrain are attained, the location of which are well removed from the site of peak normal strains. This is a critical advancement in our analysis, as bones do not normally fail under compressive strains, but strains due to bending and torsion. By calculating the shear strain distribution, we will be able to correlate the predominant locations of the stress fracture lesion to not only normal strains, but shear strains. As the yield to failure in shear is approximately 40% that of compression, much smaller shear strains could have a very significant and deleterious effect on the structural integrity of the skeleton.

A final, and unique, calculation derived from the raw strain recordings is the product of normal and shear strains to create the "strain energy density" (fig. 1c). This analysis will accent the locations in the bone that are subjected to the highest (and lowest) magnitudes of deformation.

In figures 1a through c, the complete mechanical milieu generated on the cortex is demonstrated for a specific time period in a stride taken at a certain speed. It is most interesting that the areas of greatest normal strain, shear strain and/or strain energy density in the horse cannon bone are also the areas of the cortex that have the <u>least</u> remodeling acti ity. Indeed, the most common quadrant in which stress

fracture lesion appears, the Antero-lateral cortex, is the area of the cortex with the least strain, normal, shear or strain energy density. What this extensive analysis has provided, therefore, is further support for our initial observation of the presence of the stress fracture lesion at the site of lowest normal strain. That it also appears in an area of small shear and "total" strain would suggest that the stress fracture lesion is not a product of accumulated damage or material breakdown, but is a by-product of accumulated damage or material breakdown, but is a by-product of a loading environment that sees high cyclic loads, with the pathology occurring at sites of the smallest deformations.

iv) Metabolic fatigue activity: A primary objective of the locomotion experiments is to correlate the exercise induced changes in the musculoskeletal system (mechanical), to some parameter of the respiratory/cardiovascular system (metabolic). Our original hypothesis was based on the presumption that metabolically fatigued muscle groups would engender new recruitment patterns, thereby causing a new strain distribution in the bone, subsequently stimulating a remodeling response.

Specifically, a series of metabolic stress tests were performed concurrent to the strain gage recordings. These tests produce information relating to oxygen consumption, heart rate, respiratory quotient and venous lactate concentration as a function of speed in the exercise program. During these tests, respiratory gas measurements are made and blood samples are drawn while the animal is exercising in a standardized protocol on a treadmill. Analysis of oxygen consumption and carbon dioxide

production allows the determination of maximum oxygen consumption ($VO2_{max}$), an objective indicator of maximum aerobic power. $VO2_{max}$ is defined as the maximal amount of oxygen that can be taken in and consumed for metabolic processes, and is an ideal indicator of an organism's maximal work capacity. The correlation of oxygen consumption and carbon dioxide production measurements to ventilation rate and blood lactate levels allows the determination of the anaerobic threshold. The relationship between the anaerobic threshold and maximum aerobic power can be used as a measure of the subject's degree of fitness, and therefore the capacity to "avoid" metabolic fatigue. Pre-surgery stress tests were made to ensure that the operation itself did not "create" metabolic misinformation; i.e., to obtain the pre-op speed at which peak values for the above "fatigue" parameters would be obtained (9.0 m/s). As shown in figure 2a, the decline in oxygen consumption is a result of the horse being unable or unwilling to continue to run unassisted (and may, at this point, be held in position by the lead ropes). This point of "functional fatigue" occurs with corresponding maximal oxygen consumption, maximal heart rates, RQ values above 1.2, and elevated lactate concentrations (figures 2a, b, c & d). These are all considered classical indicators of metabolic fatigue, and were used in this study, concurrent to the strain recordings, as our "flag-point" for poor coordination.

The metabolic and mechanical parameters were monitored simultaneously while the animal ran through a "standard" metabolic fatigue test. This entails one minute interval training at 4.5, 5.5, 6.5 and 9 meters/sec. running on a 10% incline on a motorized treadmill. The normal and shear strain

distributions, as well as strain energy density, were calculated through the stride as a function of increased speed and accumulating metabolic fatigue.

To demonstrate the mechanical milieu of the cannon bone both before and after metabolic fatigue, an "averaged" stride is given, and three points through that stride calculated. These plots represent the loading of the bone, the peak load, and the unloading of the bone. Fig. 3a represents the averaged stride at a speed of 6.5 m/s, taken near the beginning of the fatigue test. Fig. 3b represents the average stride at a speed of 9.0 m/s, following the onset of metabolic fatigue based on oxygen consumption (see fig. 2a). Fig. 4a, b and c represent the onset of loading for the pre-fatigue conditions (site 31%), while 5a, b and c represent the same point in the stride for the higher speed and greater fatigue conditions. Fig. 6a, b and c represent the "peak" loading conditions for the pre-fatigue condition (site 41%), while fig. 7a, b and c demonstrate the distributions of normal strain, shear strain and strain energy density for the peak load conditions of the post-fatigue exercise. Finally, the unloading conditions (52% into stride) are shown for the pre-fatigue (8a, b and c) and post-fatigue (9a, b and c) conditions.

By comparing these graphs, the object is to identify altered mechanical loading of the bone. Even with this rigorous mechanical and metabolic analysis of the bone during exercise, there are only very subtle changes which occur within the physical milieu of the bone. It would be very difficult indeed to establish these "changes" as real, rather than only slight deviations in the average stride.

Under conditions of greater speed and accumulated

indications of metabolic fatigue, we conclude that there are no apparent "alterations" in the manner of loading of the bone. Therefore, this data would suggest that the appearance of the stress fracture lesion, which occurs in that area of the cortex which is subjected to only the slightest strains, is remodeling to a stimuli other that none of an altered loading pattern. This is certainly remodeling, but apparently not one driven by adaptation to a new stimulus.

Interestingly, it would appear that the stress fracture lesion occurs in the area of the cortex where there is an interface in the shear direction. Perhaps this area of the cortex is most subject to tensile tearing of the tissue, and that accumulation of "damage" is indeed very much cycle dependent. It is important to emphasize that the material milieu at this location is well below any threat of yield failure, but that something relative to the manner of loading stimulates this remodeling response. In other words, the failure would not be due to a material breakdown, but a cycle dependent tissue "response" to the loading environment. For instance, under this mechanical shear interface, it could be proposed that the tissue is "pulling apart" and "dehydrating" at this point, i.e. Loading causes fluid to flow away from this low strain condition, decreasing tissue viability.

In summary, the lesion predominates where the normal strain, sear strain, and strain energy density is smallest. Metabolic fatigue does not induce a new loading environment, such that the remodeling that is evident in the stress fracture lesion does not appear to be "adaptive", i.e., to "new" loading conditions. Finally, the lesion appears at the tensile shear

interface, and the appearance of the lesion is cycle dependent.

Therefore, we would conclude that metabolic fatigue, in and of itself, does not create a new loading condition and therefore, stimulate adaptive behavior. It does appear, however, that something about that specific cyclic loading environment, that has little to do with fatigue itself, stimulates remodeling.

v) Validation of treadmill running: This experimental protocol depends on the mechanical milieu engendered by treadmill running being representational of the variety and intensity of "true" exercise performed in the field. To address the limitations of treadmill exercise as a realistic representation of field maneuvers, we have begun experimentation on a racetrack, with the instrumented animal running, with jockey, through an exercise regimen. We are currently able to record directly from seven of the nine channels of strain, via a small (0.7 kg.) wheatstone bridge amplifier and recording system worn by the jockey. Analog recordings were made while the animal is run on the track, and the FM recordings translated through an A $\rightarrow$ converter to calculate normal strains.

We have successfully completed the exercise and recording protocols from two horses thus far. The analysis is in progress. Our initial review of the magnitudes of normal strains, as well as its distribution, suggest that it is not significantly different from the data derived on the treadmill. This data will be the first true comparison of the similarities of the mechanical milieu of treadmill vs. field running.

b) Remodeling experiments:

The primary objective of this series of experiments is to refine an established protocol which monitors remodeling activity in bone generated by controlled mechanical stimulation. The investigators contributing to this protocol include Drs. Rubin, McLeod and Bain, as well as consultant support by Drs. Lanyon and Brand. Technical support is provided by Ms. Kinney.

i) Remodeling in response to excessive cyclic loading

In order to investigate the etiology of stress fractures induced by high repetitions of a uniform exercise regime, we utilized the functionally isolated in vivo avian wing preparation previously reported (Rubin and Lanyon, JBJS, 66A:397-402, 1984). Intermittent loads from a modified Instron machine were applied to the functionally isolated ulna via its transfixing pins. The loads of the servohydraulic actuator were adjusted to produce peak longitudinal strains at the midshaft of 1000, 2000, 3000 or 4000 microstrain. These levels of strain had been determined to be within the physiologic range when compared to strains measured from a variety of bones during vigorous activity (Rubin and Lanyon, JEB, 101:187-211, 1982). The applied strain waveform is sinusoidal with a maximum loading and unloading rate of 50,000 microstrain/sec., also physiologic in magnitude. Each ulna preparation was subjected to regimes up to as many as 30,000 cycles/day, over a single period of loading, for five days/week. Loading was discontinued either when the animals showed discomfort or at 8 weeks, whichever was the sooner.

Previous experiments, using a similar experimental preparation, demonstrated that the maximum effect of an osteogenic mechanical stimulus was generated following only a very short exposure to an intermittent load regime (thirty-six

load reversals, Rubin and Lanyon, 1984). However, static load regimes, applied at similar strain magnitudes, were ignored as an osteogenic stimulus, and osteoporosis was generated (Lanyon and Rubin, J. Biomech. 17:897-906, 1984). Following these observations, the avian wing preparation was loaded for 100 cycles/day to determine the role of strain magnitude as an osteogenic stimulus (Rubin and Lanyon, Calc. Tiss. Intl., 37:411-417, 1985). The amount of new bone formed was directly proportional to the magnitude of the engendered strain. This dose-response phenomenon produced essentially no new bone at 1,000 microstrain, yet was sufficient to inhibit the osteoporotic response. A 15-23% increase in bone cross-sectional area was stimulated at 2,000 microstrain, and a 28-42% increase in new bone at 3,000 microstrain. These experiments emphasize that the osteogenic stimulus can be triggered by very few cycles of a DYNAMIC load regime, but that the stimulus does not necessarily have to be hyperphysiological.

Surprisingly, a similar increase in bone cross-sectional area was elicited in the 30,000 cycle/day series, suggesting that the cells responsible for new bone formation had been stimulated to work at their peak capacity by exposure to the first "few" loading repetitions.

However, while microradiographs of the ulna midshaft from the 100 cycles/day animals demonstrated the degree of intracortical remodeling series to be very low, in the 30,000 cycles/day series the intracortical remodeling was extensive. This would suggest that two separate and distinct mechanisms exist; one responsible for bone modeling (new bone formation), and one for bone remodeling (intracortical tunneling).

Interestingly, the large structural defects which were apparent in the bone cortices were NOT cracks or microdamage, but rather the area of rarefaction consisted of resorption spaces and expansion of vascular channels. These spaces, combined with the exuberant periosteal new bone formation with which they were associated, presented a remarkably similar appearance to naturally occurring stress fractures (Rubin, et. al., 5th ESB, p. 230, 1986). In support of this "no damage" observation, the region of the cortex which contained the most consistent, extensive intracortical remodeling was not that subjected to the greatest strain (the area of the cortex most likely to accumulate microdamage), but rather was located about the bone's neutral axis, the area of the cortex subjected to the SMALLEST normal strains (fig. 10a).

Using the rigorous mechanical analysis developed for the cannon bone study, it is possible to calculate not only the normal strain distribution, but the shear strain distribution and strain energy density (fig. 10b & c). This analysis emphasizes that the location of the stress fracture lesion in the bird ulna, as in the horse cannon bone, appears within the area of the smallest strain deformation, not the ones of high magnitude. As in the horse study, the stress fracture lesion appears at a site of minimal shear and strain energy density.

We are continuing the high repetitive cyclic loading protocol, at 30,000 cycles per day at 2000 microstrain. We have surgically prepared twenty-two birds during the two granting years, eight of which have been loaded to at least four weeks (600,000 cycles). The resultant remodeling response to this loading regimen is being evaluated in a number of different ways,

including Finite Element Modeling (FEM), Computer Aided Tomography (CAT), and Cellular Kinetic Stereology (CKS).

ii) Remodeling in the immature skeleton:

In addition to remodeling experiments in the skeletally mature birds, we have begun a series of cyclic loading experiments in young, growing animals. This protocol should emphasize any similarities and/or differences in the remodeling/modeling process and/or stress fracture pathophysiology that may occur in a skeleton that is not quiescent. This experiment may accentuate any conditions in the young active skeleton which might either accelerate or attenuate deleterious remodeling.

Nine 4-month old turkeys have been surgically prepared, as above, and are currently being subjected to the applied loading regimes. Following their one-month loading protocol, they will be euthanized and the bones analyzed for cell mobilization, degree of mineralization, location of new bone formation and sites of intracortical resorption.

iii) FEM activity: A major limitation to the cyclic loading protocol is the large investment of man hours per resultant section. Essentially, only one "data point" is generated for every eight weeks of time invested in the ulna protocol. We are developing an FEM model of the ulna such that more data can be extracted from each experimental section. What this entails is generating a three dimensional model of the entire ulna, via sequential transverse sectioning of the ulna. These sectional properties are then digitized, and the longitudinal model

constructed. By modeling the ulnas in this manner, the periosteal strain gauge reading can be incorporated into first-order "strength of materials" considerations, and computational strain predictions made throughout the corresponding longitudinal mesh sites. This will allow us to predict full stress and strain field data for the turkey model, and therefore correspond the remodeling changes throughout the ulna to the applied strain environment, yielding significantly more data per animal.

To more accurately quantitate the remodeling response observed in the ulna specimens, we are currently developing a video image analysis system based on our Dual Computer System and image processing boards. This will facilitate accurate measurement of the ulna, especially as we intend to use many more sections with the F.E.M.

The reconstructed turkey ulna, which can now be viewed from any orientation (fig. 11a, b & c) allows us to input the known load data onto the osteotomized surfaces of the bones, and adjust the assumptions at the nodes until the gaged section matches the recorded strains. The FEM is made up of 18 layers of elements between the pins, and is contoured in a regular 80 x 80 array, for which the strain at each site is determined from an inverse-distance-squared-weighted-average of the stains computed for 320 Gauss points in the finite element mesh. This allows us to break up each contoured section into 40 different areas, (fig. 12), and then compare the calculated neutral axis to that determined by mechanical testing.

Most importantly, we are developing the F.E.M. model to represent the post-remodeling phase, and to correlate sites of new bone formation to the specific character of the mechanical

milieu (fig. 13). This is the first time a controlled remodeling experiment can be used to validate a theoretical model. We are currently developing this FEM based theoretical "predictor" of remodeling, to use the model to identify those areas of the cortex at greatest risk of failure, and the activities which accelerate the deleterious behavior.

iv) CAT activity: To facilitate the sectioning of the entire ulna, we have attempted to generate the sectional properties using CAT scanning. Our first attempts have entailed the use of a G.E. 8800 CT scanner, but the resolution of the system was inadequate to simultaneously differentiate new bone formation and intracortical remodeling. In collaboration with the University of Michigan, Department of Orthopaedic Surgery, and the Ford Motor Company, we again attempted to map out the remodeling response using their high resolution micro-CAT scan. Unfortunately, again the resolution was insufficient to optically identify intracortical remodeling activity. We have concluded that physical cross-sections are required.

v) CKS activity: Standard histology is being continued, and a program of Cellular Kinetic Stereology (CKS), is being developed by Dr. Steven Bain, a post-doctoral fellow in the laboratory. Dr. Bain is trying to quantitate periosteal and endosteal changes in cellular activity following only very few days of loading. This may indicate how the stress fracture lesion is instigated. It may also provide a means of more quickly identifying deleterious load regimens.

Summary

The etiology of the stress fracture condition has been considered previously to be the result of accumulative fatigue damage. However, the controlled loading environment made possible by the avian preparation suggests that the most extensive and demonstrable bone defects associated with excessive repetitions of cyclic loading are not those resulting from an actual "fracture" across the cortex, but rather a large area of rarefaction, resulting from resorption, inevitable leading to pain and structural failure. Based on the horse and turkey experiments, we believe the resorption of the cortex, and the eventual failure caused by the decrease in effective load-bearing area, are more likely to be a cycle dependent, strain engendered remodeling response within the bone "tissue", rather than a failure of the bone "material" due to fatigue.

The following points are particularly noteworthy:

1. Location of the lesion: The "stress fracture" lesions produced by externally loading the avian preparation do not occur in the area of the cortex subjected to highest strains; rather these lesions occur predominantly about the bone's neutral axis of bending where the strains are least. This is supported by calculating the distribution of shear strain and strain energy density. Once again, the lesion occurs in the area of least strain. Interestingly, this is also an area of a shear "interface", where the direction of shear changes causes a "tearing". Transposition of our strain gauge data, derived from both human tibia and horse metacarpal, demonstrates that naturally occurring stress fractures in the human and equine will also occur close to the region of the neutral axis, where

deformation is least.

2. Degree of mineralization: In both the natural and artificially induced lesions, there is subperiosteal and subendosteal new bone formation adjacent to the most marked intracortical lesion. These lesions were commonly characterized by a defect of less well mineralized tissue stretching from one surface of the original cortex to the other. As demonstrated by microradiography, these lesions were not distinct cracks, but rather a large area of rarefaction caused by substantial intracortical remodeling.

Considering the presence of new bone adjacent to the areas of porosity within the cortex, as well as the apparent absence of extracellular "damage", this "stress fracture" lesion should not be considered a distinct failure of an inanimate material. Rather it is a remodeling response of a live viable tissue to a distinct new functional strain situation, the CONSEQUENCE of which is the "stress fracture" lesion. This alternative etiology is supported by the onset of the stress fracture lesion occurring in boot camp cadets during the second and third weeks of training. While 14 days is inadequate time to accumulate sufficient loading repetitions to engender a "paper clip" failure, this period is concurrent with the time in which bone, stimulated to remodel, will have resorbed as part of the remodeling process, but will not yet have mineralized enough to afford any structural support to the otherwise weakened cortex.

It is important to emphasize that our original hypothesis, that metabolic fatigue will instigate a new loading environment and subsequently stimulate adaptive remodeling, does not appear

to be supported by these experiments. Even under sever indications of metabolic fatigue, new loading conditions are not precipitated. Indeed, as the stress fracture lesion appears at the site of least normal strain, and this site does not change, a stimulus to remodel does in fact accumulate, but in no way is dependent on accumulated damage. Indeed, because of the low strains involved, we are now certain that the skeletal material itself is not responsible for the failure. Instead, it would appear that this remodeling is a cycle dependent cellular response to a specific mechanical milieu, but not one stimulated by adaptation, nor strains in a damaging range. We propose that something about the mechanical milieu at that point in the bone diminishes the bones viability and accelerates resorption, but in no way is dependent on high levels of material strain.

We realize that these data contradict the general definition of stress fractures (i.e., a result of fatigue), and we are confident that a new etiology must be considered. Because of the location of the lesion, close to the neutral axis of bending and not in the area of the cortex subjected to the highest strains, we believe the lesion NOT to be a "fatigue fracture", but rather a cycle dependent remodeling consequence to a new loading environment. This alternative etiology of stress fractures could explain an array of poorly defined musculoskeletal complaints associated with normal radiographic appearance. While purely speculative at this time, it is possible that some cases of "shin splints", heel pain, and "groin pull" represent early stages of bone resorption as described above, with remodeling and "repair" occurring before radiographic findings.

We believe the experimental protocols currently underway (locomotory and remodeling) will provide new insights into the pathophysiology of the stress fracture lesion, as well as indicate mechanisms to minimize their occurrence.

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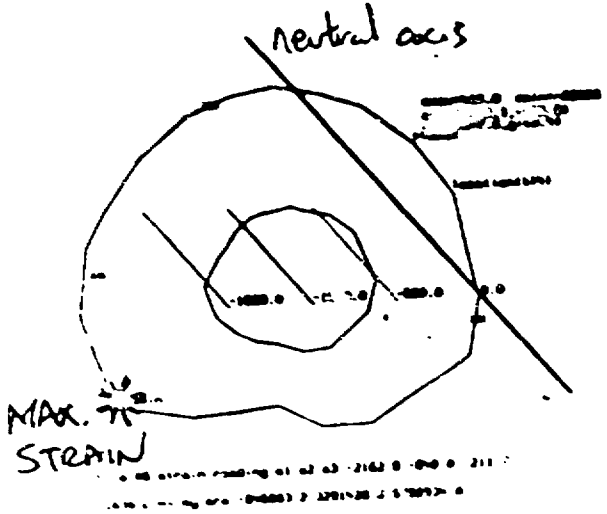

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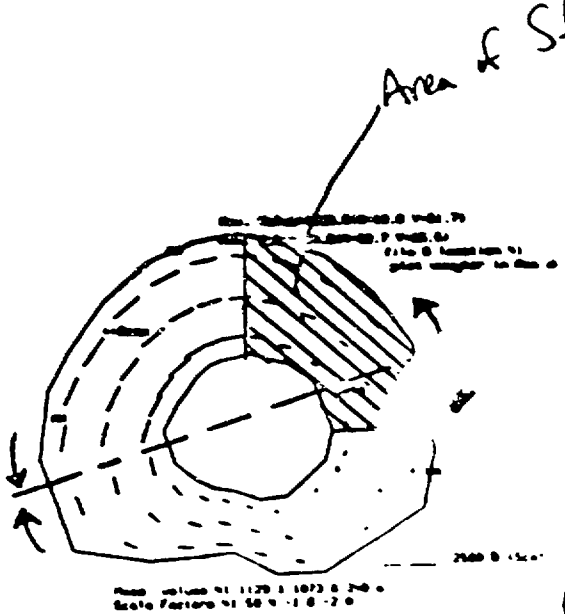


Fig. 1b

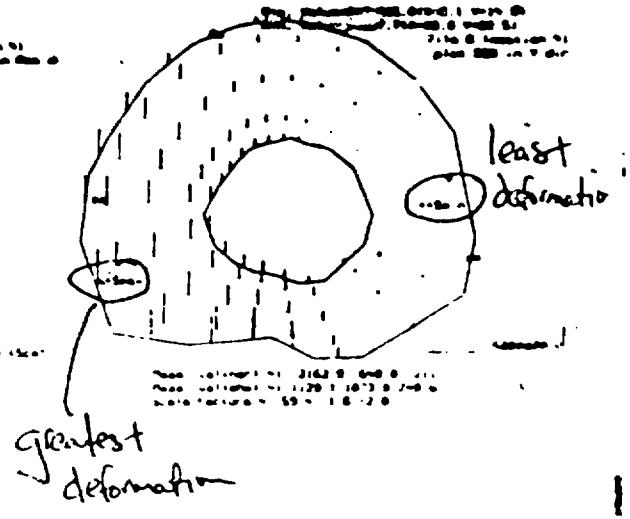


Fig. 1c

EXERCISE STRESS TEST (aerobic component)
and
TIME TO FUNCTIONAL FATIGUE

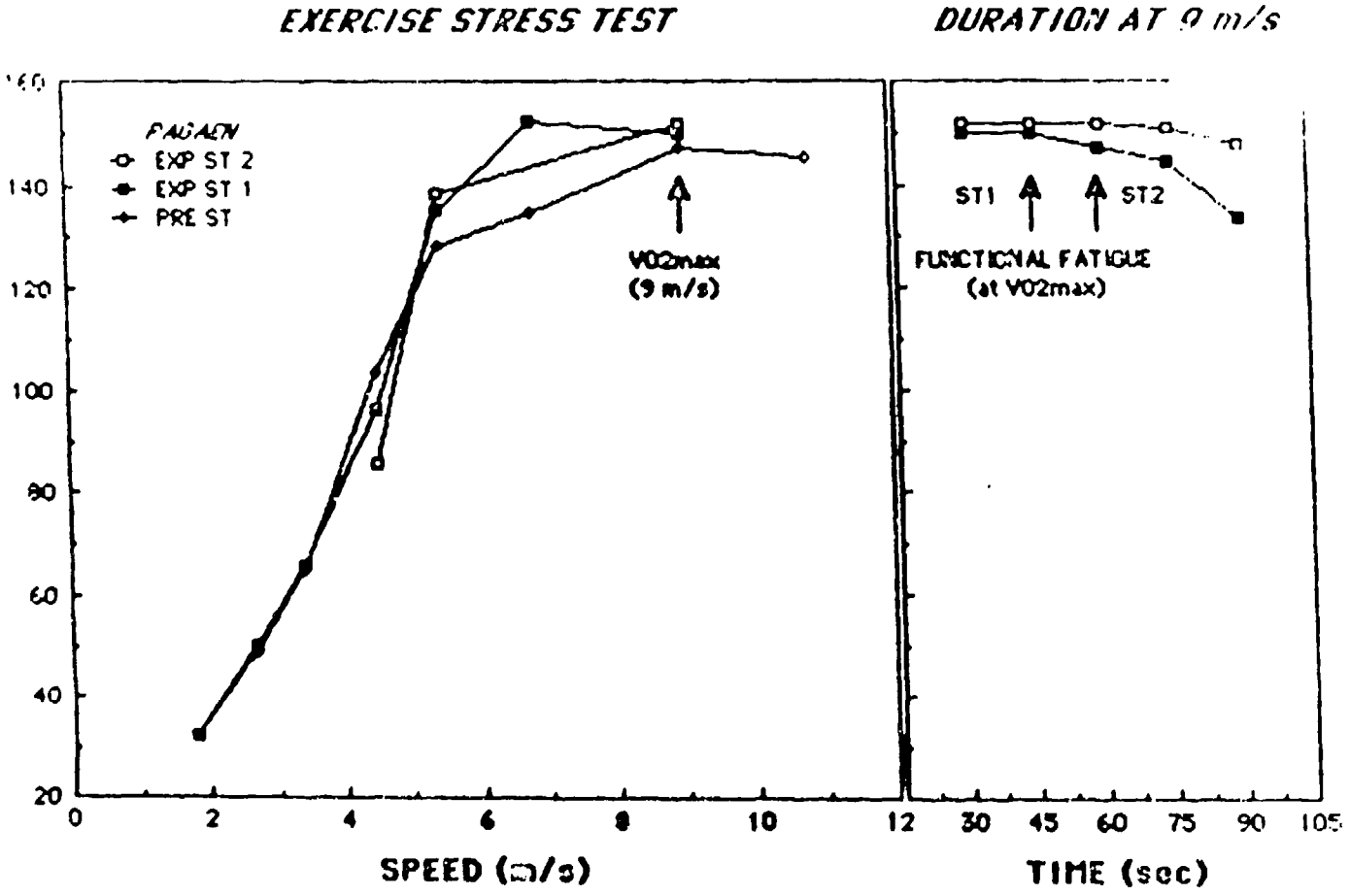


Fig. 2a

EXERCISE STRESS TEST

HEART RATE VS SPEED

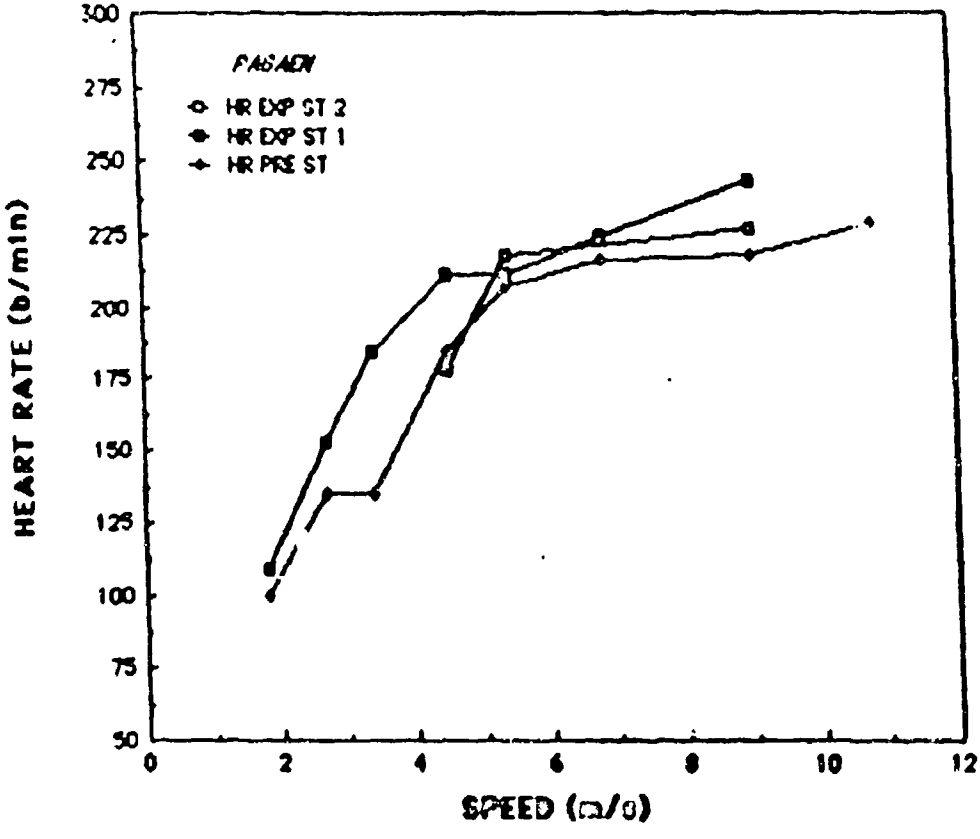


Fig. 2b

EXERCISE STRESS TEST

VENOUS LACTATE CONCENTRATION VS SPEED

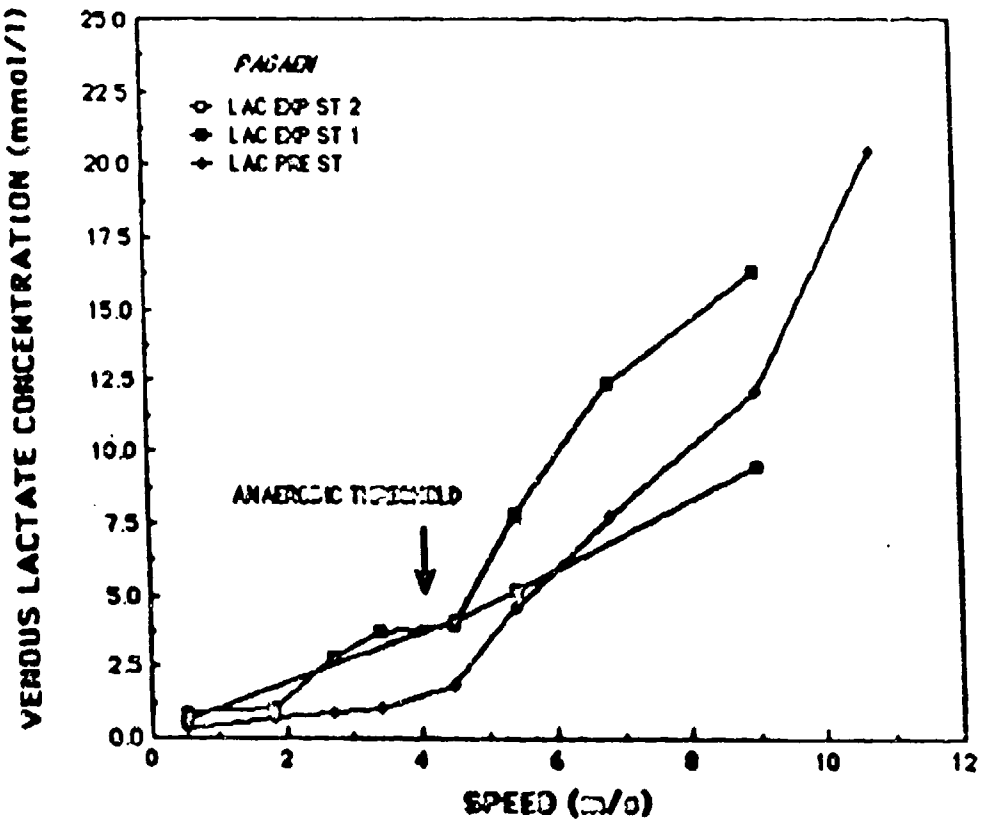


Fig. 2c

EXERCISE STRESS TEST

RESPIRATORY QUOTIENT VS SPEED

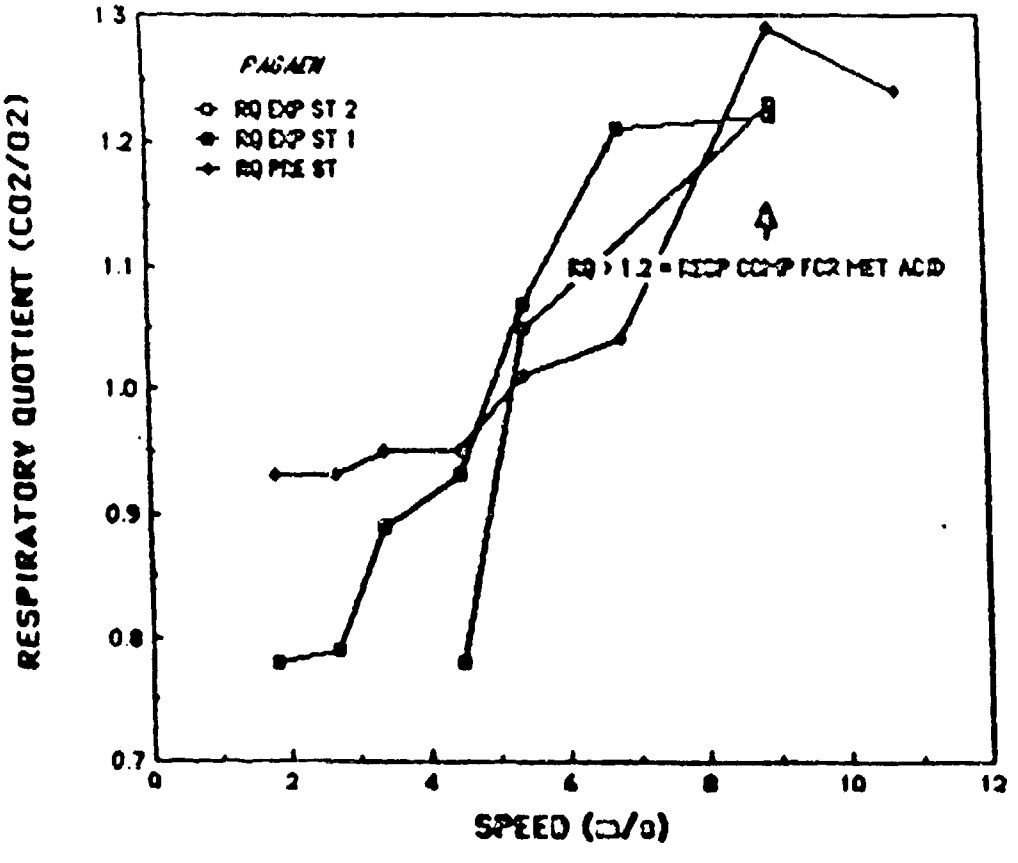
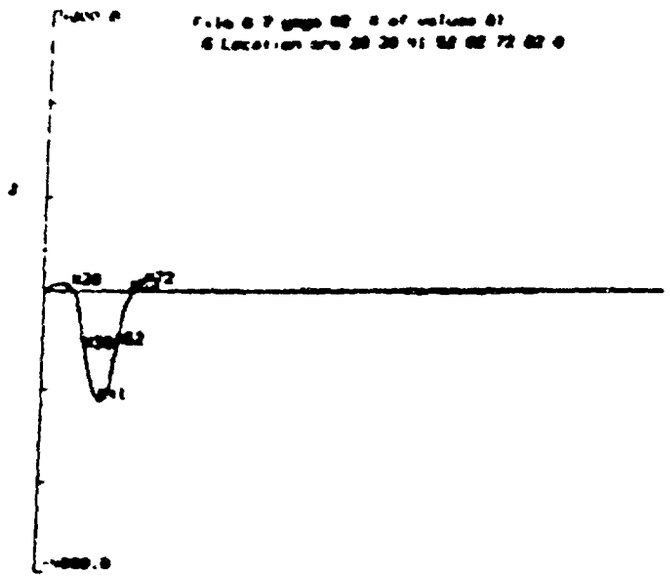


Fig. 2d



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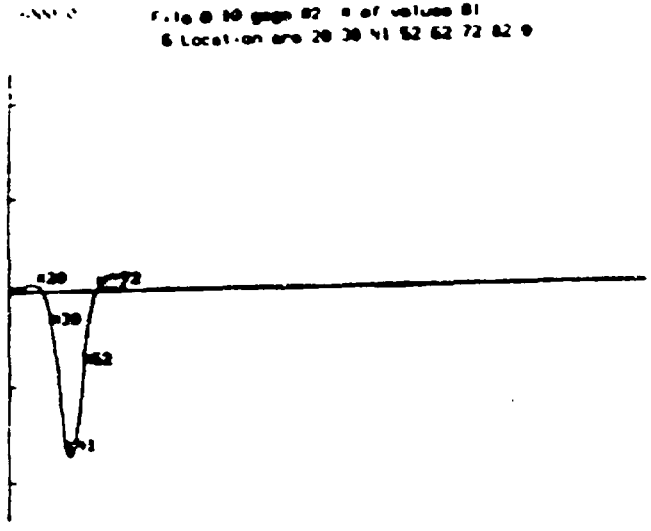
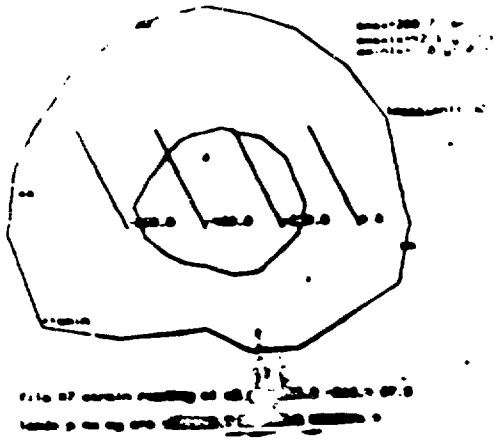


Fig. 3b



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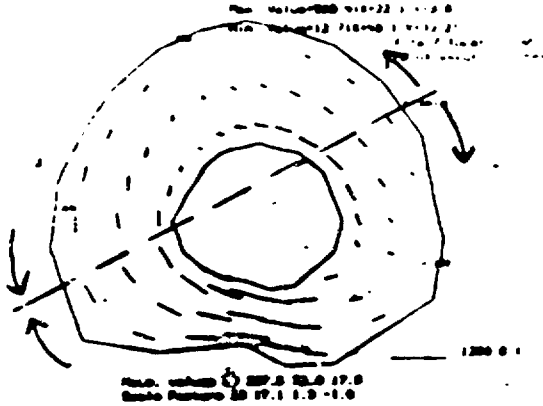
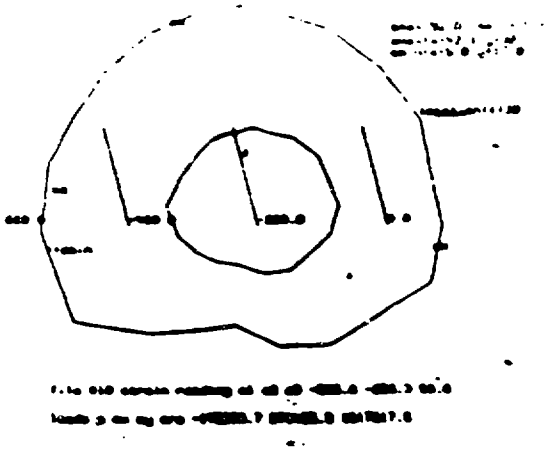


Fig. 4b

Fig. 4c



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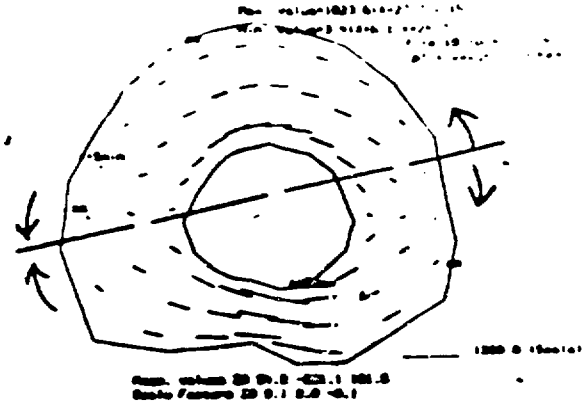
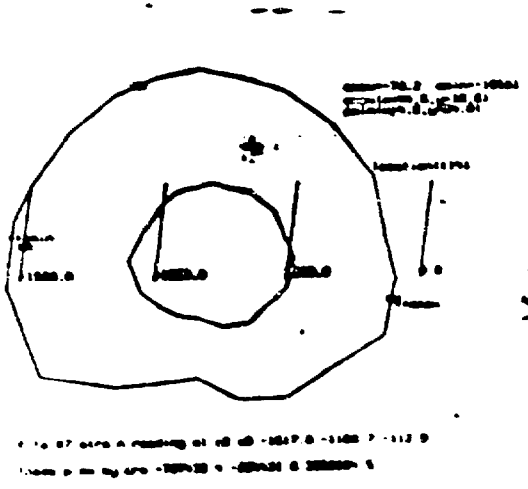


Fig. 5b

Fig. 5c



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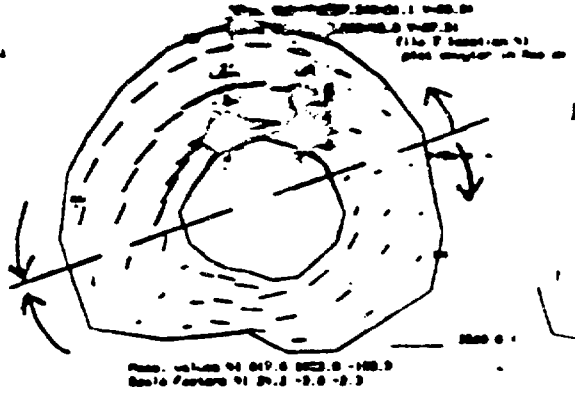

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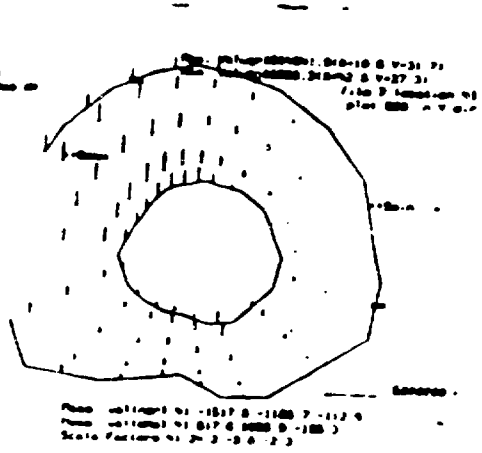

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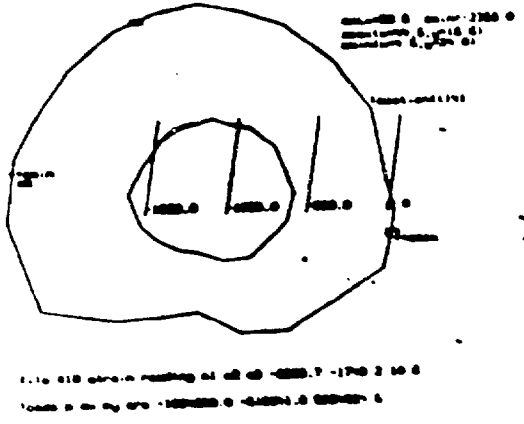

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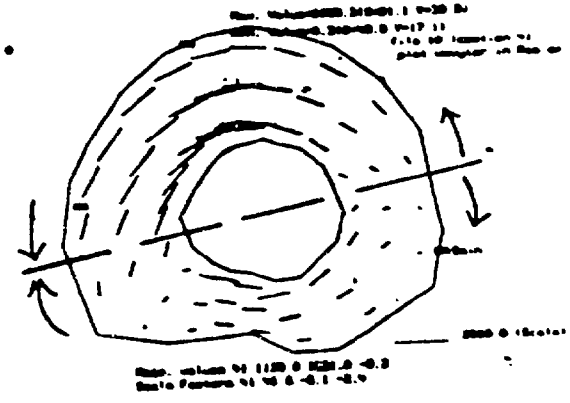

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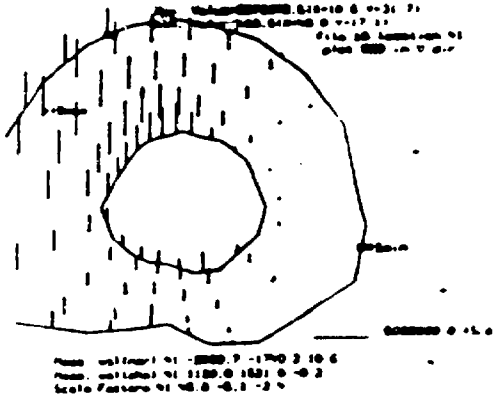

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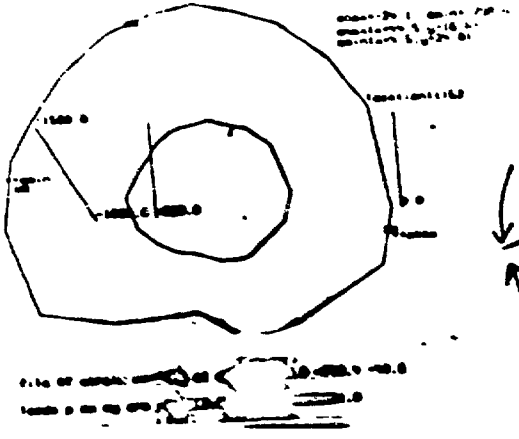

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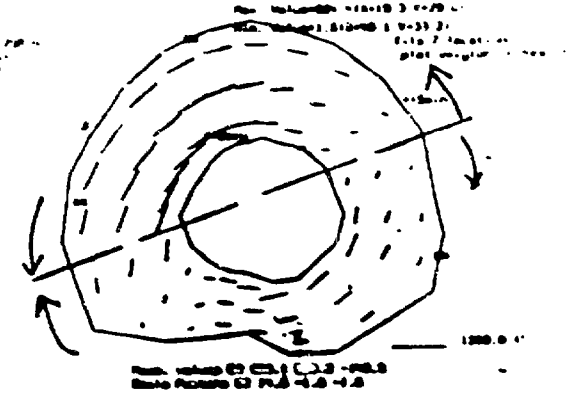

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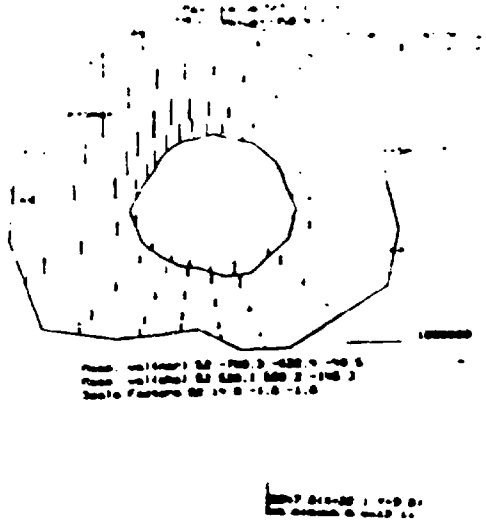

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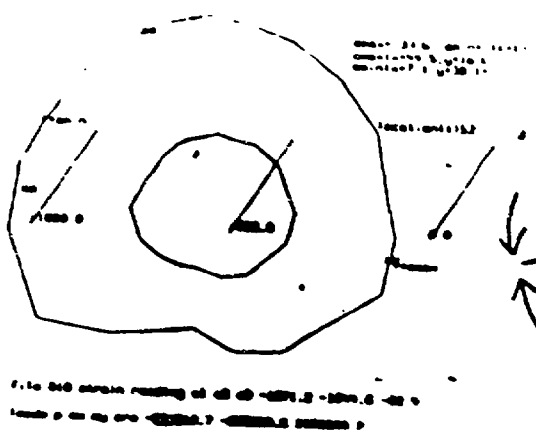

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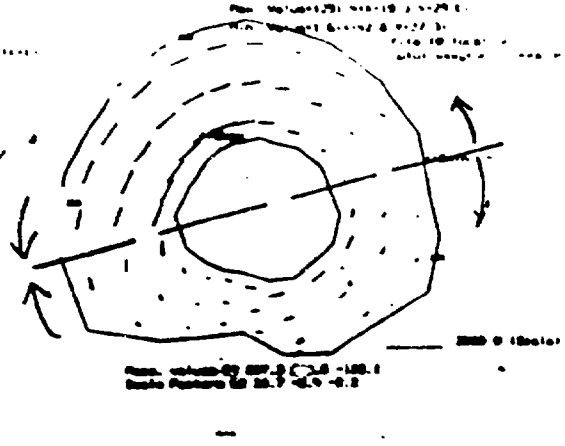

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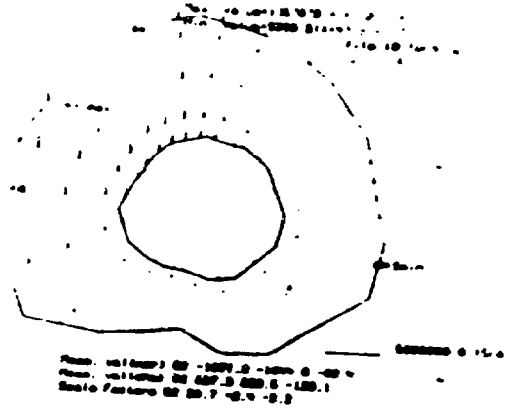

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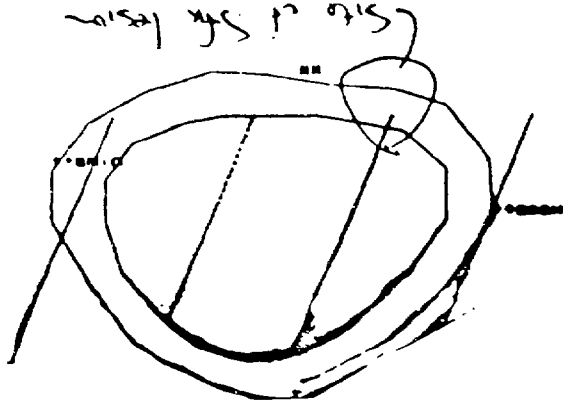

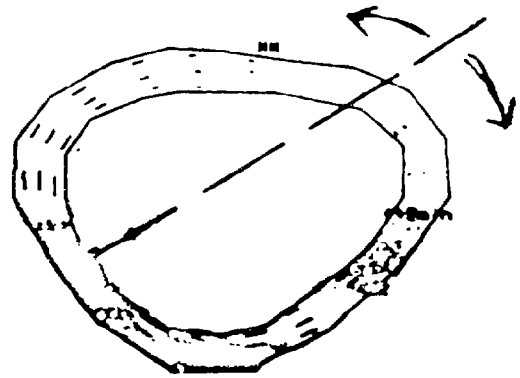

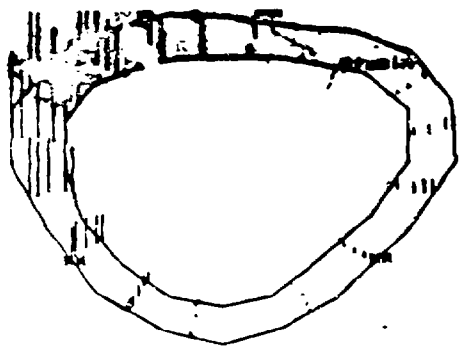

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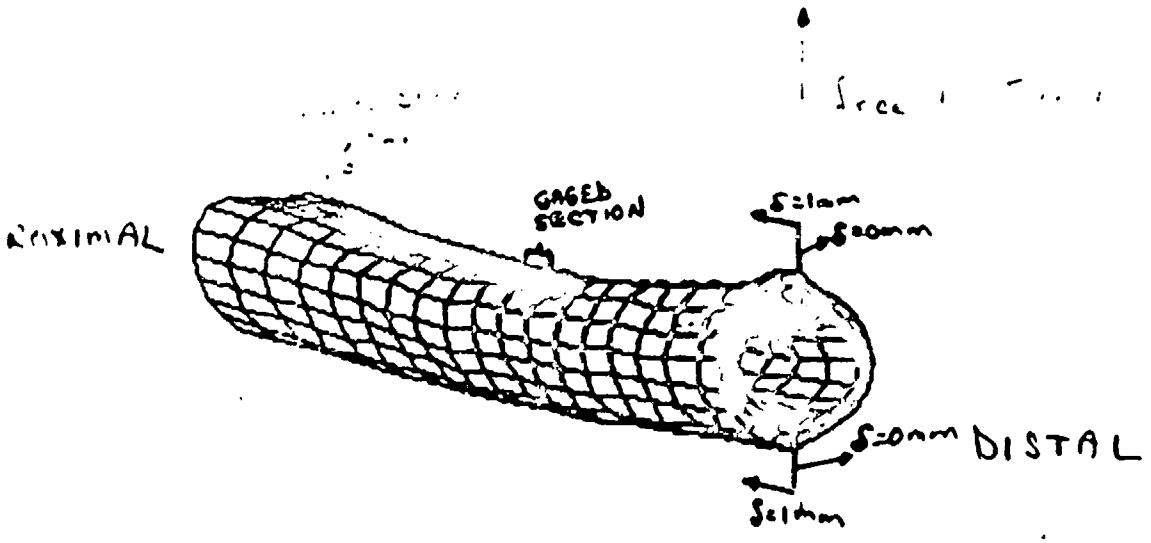

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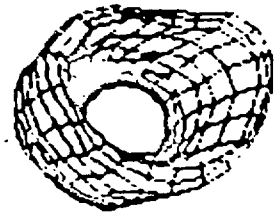
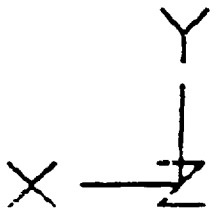


VIEWED FROM
PROXIMAL END



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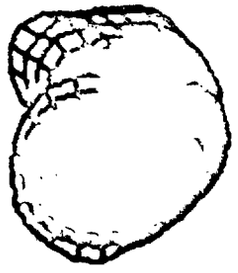
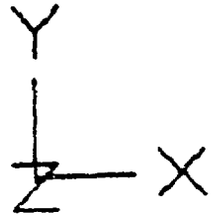


LOWER-OBLIQUE VIEW



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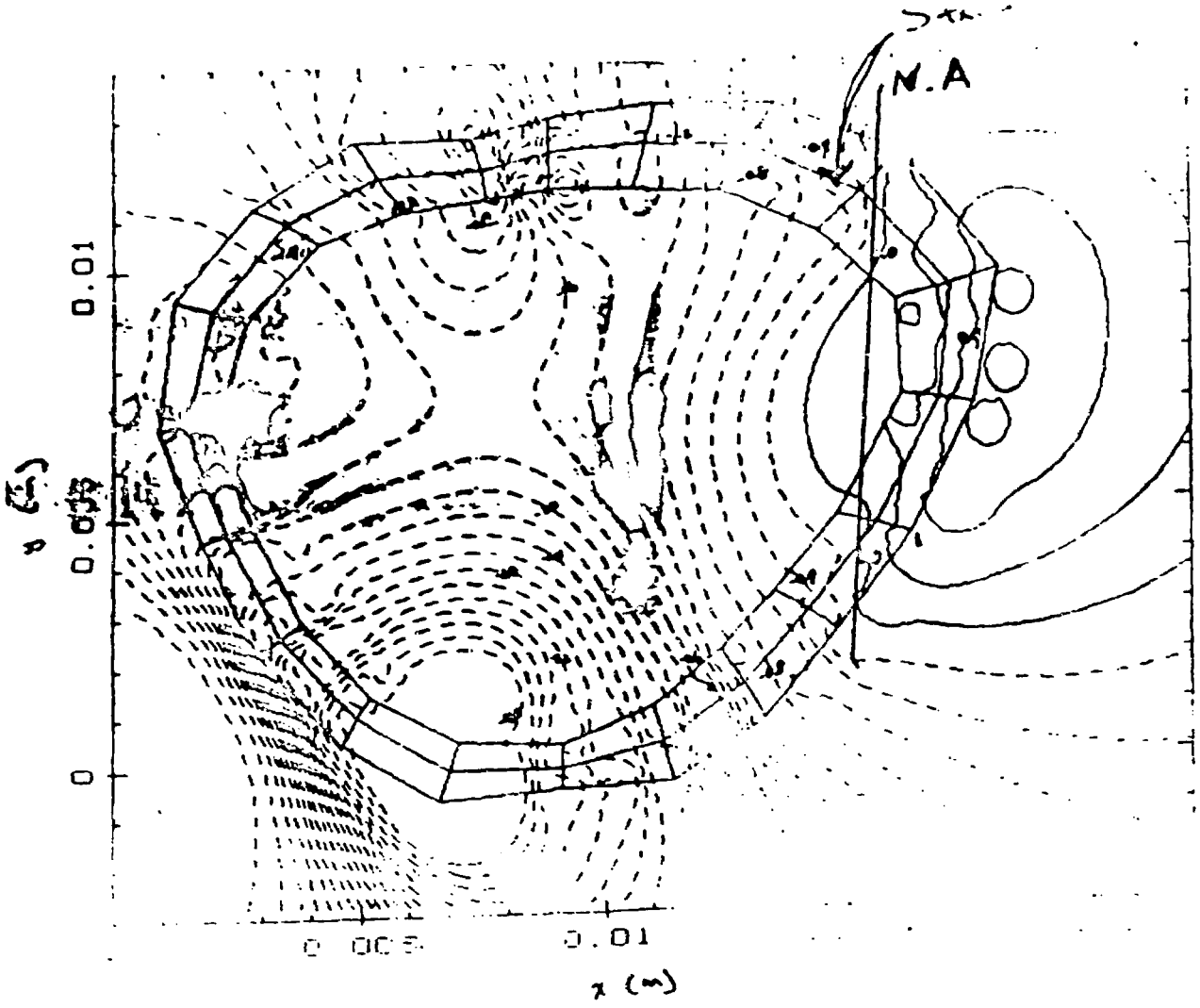

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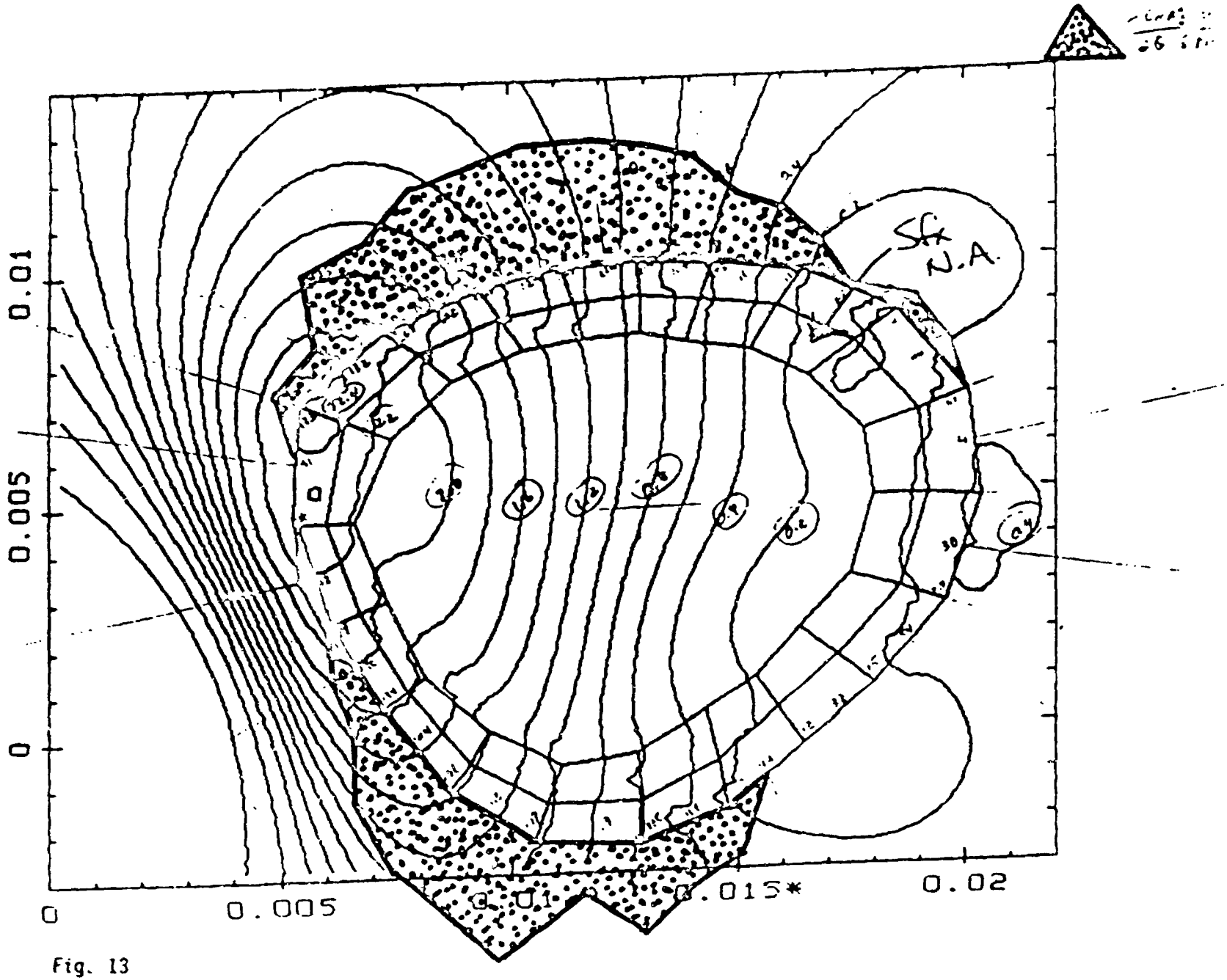

Fig. 13